Age, Vol. 21, 153-173,1998

INTERVENTION IN THE AGING IMMUNE SYSTEM: INFLUENCE OF DIETARY RESTRICTION, DEHYDROEPIANDROSTERONE, MELATONIN, AND EXERCISE

Mohammad A. Pahlavani
Geriatric Research, Education and Clinical Center,
South Texas Veterans Health Care System and
Department of Physiology,
University of Texas Health Science Center,
San Antonio, Texas 78284

ABSTRACT

The decline in immunologic function with age is associated with an increase in susceptibility to infections and the occurrence of autoimmune diseases and cancers. Hence, the restoration of immunologic function is expected to have a beneficial effect in reducing pathology and maintaining a healthy condition in advanced age. A number of therapeutic strategies have been employed to intervene in the aging immune system. This article reviews the effect of dietary restriction (DR), dehydroepiandrosterone (DHEA) treatment, melatonin (MLT) therapy, and exercise on modulating the immune responses and retarding/reducing immunosenescence. DR has been subject to intensive research and is known to be the most efficacious means of increasing longevity, reducing pathology and enhancing immune function.

The circulatory levels of the androgenic hormone DHEA and the pineal hormone MLT decrease with increasing age, and this decrease has been correlated with the age-related decline in the immune system. Therefore, the observation that immunosenescence is associated with low levels of DHEA and MLT has provided a rationale for therapeutic intervention. DHEA treatment and MLT therapy both exhibit immuno-stimulatory actions and preliminary reports indicate that hormonal (DHEA or MLT) substitution therapy reverses immunosenescence in mice. Similarly, exercise in some studies has been shown to enhance the immune response. However, these findings have not been confirmed by other laboratories. Thus, at the present time, it is difficult to draw any definitive conclusions on the efficacy of DHEA, MLT, and exercise on reversing or restoring the aging immune system.

All correspondence should be addressed to:
Mohammad A. Pahlavani, Ph.D.
GRECC (182)
Audie Murphy VA Hospital
7400 Merton Minter Blvd.
San Antonio, TX 78284
Telephone No. 210/617-5197
Fax No. 210/617-5312
E-Mail: Pahlavani@uthscsa.edu

INTRODUCTION

The age-related decline in the immune system has been extensively documented in humans and in rodents (reviewed in 1-5). Of clinical importance is the observation that the decrease in immunologic function with age is accompanied by an increase in incidence of various age-associated diseases, e.g., infections, cancer and certain types of autoimmunity (6,7). Therefore, the age-related changes in the immunologic response appear to be very important physiologically in mammals. Evidence from both humans and rodents show that the T cell-dependent immune functions are more sensitive to aging than other immune cells. Defects in T cell proliferative capacity/responsiveness, cytotoxicity, interleukin-2 (IL-2) production and receptor expression, calcium signal generation and signal transduction are frequently cited problems observed with immunosenescence.

Because the immune system plays an important role in aging and because the decline in immune status may be linked to the development of many age-associated diseases (8), a number of intervention strategies have been proposed to prevent the age-related loss of immune function in old animals. These intervention methods include transplanting various immune cells from young donors into old recipients (9-12), IL-2 therapy (reviewed in 13), hormonal therapy, e.g., thymic hormone (14,15), androgenic hormone DHEA (16,17), pineal hormone MLT (18,19), nutritional regimens (20,21), and exercise (22-24). In addition, recent studies by Chandra (25) and Bogden et al. (26) have shown that supplementation with multi-vitamins and minerals improves the immune response in the elderly human subjects. Similarly, Meydani et al. (27) have shown that vitamin E supplementation improves the immune response in healthy elderly subjects. Among the different intervention strategies employed thus far, nutritional manipulation, in particular reduction of caloric intake has been found to be a robust tool in increasing longevity (28,29) and retarding/reducing immunosenescence (20,21,30). In recent years, a number of reviews have been written on the anti-aging effect of DR (25,26), DHEA (31,32), and MLT (33,34). The purpose of this review is to examine the current literature regarding the intervention in aging immune function by DR and hormonal (i.e., DHEA and MLT) manipulation. In addition, the potential influence of exercise, known to modulate immune function, will be discussed in relation to aging.

Influence of Dietary Restriction on the Aging Immune System

The initial study in the 1930's by McCay et al. (35) showed that severe reduction of food intake in rats increased the life span dramatically. Subsequent studies in the 1950s and 1960s demonstrated that dietary restriction (i.e., undernutrition, not malnutrition) significantly prolonged the survival of rodents (28,29). This prolongation has been observed with a variety of different techniques that reduce the amount of food consumed by rodents (36). Over the past decade, it has become apparent that the reduction in total calories is the component of the dietary restriction regimen responsible for the increase in survival (28,36,37). Numerous laboratories have shown that dietary restriction not only increases the survival of rodents, but also retards/reduces the incidence of a variety of age-associated diseases such as cancer and renal disease (30,36,37).

Because it is well documented that dietary restriction enhances longevity and reduces pathology, there has been a great deal of interest on whether dietary restriction decreases pathology through its action on the immune system (8). One of the classical tests of immune status is mitogen/antigen-induced lymphocyte proliferation. The initial study in this area was conducted by Walford's laboratory, which showed that lymphocyte proliferation in response to phytohemagglutinin (PHA) and pokeweed mitogen (PWM) was similar for control and dietary restricted mice at 4 months of age. However, at 12 and 28 months of age, lymphocytes from dietary restricted mice showed higher proliferation in response to these two mitogens (8). Several other studies by Walford's laboratory with other strains of mice confirmed the initial observation that mitogen-induced lymphocyte proliferation was greater for mice fed a restricted diet (38). Since 1974, a number of different laboratories using various strains of mice and rats have confirmed the initial observation by Walford's group that the proliferative response of lymphocytes to mitogen was greater for mice fed a restricted diet (40% reduction in calories). In some studies, the influence of dietary restriction on immunologic function was conducted in young or middle-aged animals, whereas in others the effect of DR has been documented in truly aged animals. These studies are summarized in Table 1. In the majority of the studies, polyclonal activators such as PHA, Concanavalin A (ConA), PWM, PPD (purified protein derivative), or anti-CD3 antibody, were used to stimulate lymphocytes. In some studies, however, antigens (e.g., alloantigen) were used to activate lymphocytes.

The data in Table 1 show that the immune response decreases with age and that dietary restriction reduces the age-related decrease in immunologic responses such as mitogen-induced lymphocyte proliferation, cytokine production, and antibody responses. Thus, the overwhelming majority of the studies show that dietary restriction enhances the immune response and this increase ranges from 35% to 450%. For example, in an early study, Fernandes's laboratory reported that mitogen-induced lymphocyte proliferation and IL-2 production were reduced dramatically with age in short-lived autoimmune-prone strain NZB/W mice (39). Dietary restriction significantly reduced the age-related decline in mitogenesis and IL-2 production by splenocytes from NZB/W mice. In another study, they showed that IL-2 activity, as well as IL-2 receptor (IL-2R) expression (number of IL-2R site per cell) was increased significantly in ConA stimulated splenocytes isolated from the 19-month-old F344 rats fed a restricted diet compared to the rats fed *ad libitum* (40). Using a limiting dilution assay, Miller's group found that the percentage of IL-2 producing cells decreased with age; however, this decline was less in mice fed a restricted diet. For example, 32-month-old mice fed ad libitum retained only 15% of their helper T cell function (measured as IL-2 producing cells) compared to 7-month-old control mice. In old mice fed a restricted diet, 53% of helper T cell function was retained (41). The immunoenhancing effect of dietary restriction is not only restricted to changes in mitogenesis and cytokine production, but also leads to changes in the percentage and phenotypic expression of T cells. It has been shown that the percentage of T cells ($CD3^+$), cytotoxic/suppressor T cells ($CD8^+$) and natural killer (NK) cells ($OX8^+$ $OX19^-$) were increased in 8-month-old dietary restricted Lobund-Wistar rats compared to the control rats fed *ad libitum* (42). Thus, these studies indicate that dietary restriction enhances the immune function and retards/reduces the age-related decline in immune responses in rodents.

In 1990, we reported that dietary restriction significantly increased the immune response in aged rats (43). In this study, at 6 weeks of age Fischer 344 rats were subjected to a restricted diet (60% of the diet consumed by rats fed ad libitum). After 5, 12, 21, and 28 months of age, ConA induction of proliferation and IL-2 production (activity) by spleen lymphocytes were measured. As shown in Figure 1, the proliferative response of lymphocytes to ConA in both dietary restricted rats and ad libitum fed rats declined significantly with increasing age. No differences were observed in mitogenesis and IL-2 production between dietary restricted rats and the ad libitum fed rats at 5 and 12 month of age. However, the induction of proliferation and IL-2 expression was significantly higher in 21 and 28-month-old dietary restricted rats compared to the rats fed ad libitum. In addition, we found that the increase in IL-2 activity was paralleled by an increase in the levels of the IL-2 mRNA transcript. This was the first study to demonstrate that dietary restriction alters IL-2 expression at the level of transcription.

Over the past decade, the expression of heat shock genes has been used as a model for studying the regulation of gene expression at the transcriptional level. Heat shock protein 70 (hsp70), which belongs to the HSP70 family, is the most inducible of the heat shock proteins by stress, and this protein appears to play a

Table 1. Influence of Dietary Restriction and Aging on Immunologic Function

Species	Strain	Age (months)	Lymphoid Cells	Immune Parameters	Change with Age	Change with Restriction	Ref
Rat	F344	6-24	Splenocyte	Proliferation (ConA)	Decrease	Increase	49
				IL-2 activity	Decrease	Increase	
	Wistar	3	Splenocyte	Proliferation (ConA)	———	Increase	50
	F344	5-28	Splenocyte	Proliferation (ConA)	Decrease	Increase	43
				Proliferation (LPS)	Decrease	Increase	
				IL-2 activity/mRNA	Decrease	Increase	
				IL-3 activity	Decrease	Increase	
	B.Norway	6-30	Splenocyte	Proliferation (ConA)	Decrease	Increase	51
				IL-2 activity	Decrease	No change	
				IFN-γ	Increase	Increase	
	Wistar	6	Splenocyte	Antibody (SRBC)	———	No change	52
	Buffalo	6		Antibody (SRBC)	———	No change	
	Wistar	12		Antibody (SRBC)	———	Decrease	
	Buffalo	12		Antibody (SRBC)	———	No change	
	Wistar	4-27	Splenocyte	Proliferation (ConA)	Decrease	Increase	53
	F344	4	Splenocyte	Proliferation (PHA)	———	No change	54
				Proliferation (ConA)	———	No change	
				IL-2 activity	———	No change	
				IL-2 receptor/cell	———	No change	
	F344	4-19	Splenocyte	Proliferation (PHA)	Decrease	Increase	40
				Proliferation (ConA)	Decrease	Increase	
				IL-2 activity	Decrease	Increase	
				IL-2 receptor/cell	Decrease	Increase	
	F344	6-27	Splenocyte	IL-2 activity	Decrease	Increase	55
				Cytotoxicity	Decrease	No change	
	F344	6-24	T cells	IL-2 mRNA	Decrease	Increase	48
	F344	6-24	T cells	IL-2 activity/mRNA	Decrease	Increase	56
				NFAT activity	Decrease	Increase	
Mouse	C3H/He	2	Splenocyte	Proliferation (MLR)	———	Increase	57
				IL-2 activity	———	Increase	
	C3B10RF1	2	Splenocyte	Proliferation (PHA)	———	Increase	58
				Proliferation (ConA)	———	Increase	
				Proliferation (PWM)	———	Increase	
				Proliferation (LPS)		Increase	
	C57BL/6J	8	Splenocyte	Proliferation (PHA)	———	Increase	59
				Proliferation (ConA)	———	Increase	
				Proliferation (MLR)	———	Increase	
				Proliferation (PPD)	———	No change	
				Proliferation (PWM)	———	No change	
				Proliferation (LPS)	———	No change	
	C57BL/10	8	Splenocyte	NK activity	———	Increase	60
	C57BL/6J	4	Splenocyte	Proliferation (PHA)	———	Decrease	8
				Proliferation (ConA)	———	Decrease	
				Proliferation (PWM)	———	No change	
		12	Splenocyte	Proliferation (PHA)	———	No change	
				Proliferation (ConA)	———	No change	
				Proliferation (PWM)	———	No change	
	C3B10RF1	4-28	Splenocyte	Proliferation (PHA)	Decrease	Increase	61
				Proliferation (ConA)	Decrease	Increase	
	BDF1	3-30	Splenocyte	Proliferation (CD3)	Decrease	Increase	63
				CD4+ T cell	Decrease	Increase	
				CD8+ T cell	No change	Increase	
				Calcium flux	Decrease	Increase	
	B6CBAT6F1	7-30	Splenocyte	pHTL (precursor)	Decrease	Increase	41
				pCTL (precursor)	Decrease	Increase	
	C57BL/10	3-24	Splenocyte	Proliferation (CD3)	Decrease	Increase	61
				CD4+ T cell	Decrease	Increase	
				CD8+ T cell	Increase	No change	
				Calcium flux	Decrease	No Change	

(Continued on next page)

Table 1 *(continued)*

Species	Strain	Age (months)	Lymphoid Cells	Immune Parameters	Change with Age	Change with Restriction	Ref
Mouse	B10C3F1	6	Splenocyte	Proliferation (PHA)	———	No change	62
				Proliferation (ConA)	———	Increase	
	B10C3F1	12	Splenocyte	Proliferation (PHA)	———	Increase	
				Proliferation (ConA)	———	Increase	
	B10C3F1	32	Splenocyte	Proliferation (PHA)	———	Increase	
				Proliferation (ConA)	———	Increase	
	B6D2F1	6-30	Splenocyte	Proliferation (PHA)	Decrease	Increase	63
				Proliferation (ConA)	Decrease	Increase	
				Proliferation (LPS)	Decrease	Increase	
				Proliferation (SEB)	Decrease	Increase	
				IL-2 activity	Decrease	Increase	
				IFN-γ	Increase	No change	
				CD4+pgp1-(naive)	Decrease	Increase	
				CD4+pgp1+(Memory)	Increase	Decrease	
	NZB/W	4	Splenocyte	Cytotoxicity	———	No change	66
				Antibody (SRBC)	———	No change	
	NZBXNZW	7-10	Splenocyte	Proliferation (ConA)	Decrease	Increase	39
				Proliferation (LPS)	Decrease	Increase	
				IL-2 activity	Decrease	Increase	
				Antibody (SRBC)	Decrease	Increase	
				G.V.H.	Decrease	Increase	
				Cytotoxicity	Decrease	Increase	
	NZB/W	2-11	Splenocyte	Proliferation (ConA)	Decrease	Increase	64
	NZB/W	4	Splenocyte	Proliferation (ConA)	———	No change	65
				Proliferation (PHA)	———	No change	
				Proliferation (LPS)	———	Increase	
				IL-2 activity	———	Increase	
				Cytotoxicity	———	Decrease	
				Antibody (SRBC)	———	Increase	
	NZB/W	3-11	Splenocyte	Proliferation (ConA)	Decrease	Increase	67
				IL-2 activity	Decrease	Increase	
Mouse	NZB/W	6	Splenocyte	Proliferation (PWM)	———	Increase	65
				Proliferation (LPS)	———	No change	
				IL-2 activity	———	Increase	
				Cytotoxicity	———	Increase	
				Antibody (SRBC)	———	Increase	

ConA, concanavalin A; PHA, phytohemagglutinin; LPS, lipopolysaccharide; PWM, pokeweed mitogen; MLR, mixed lymphocyte reaction; PPD, purified protein derivative; SEB, staphylococcal Enterotoxin B; IL-2, interleukin-2; IL-3, interleukin-3; IFN-γ, Interferon-gamma; SRBC, sheep red blood cell; NK, natural killer; pHTL, precursor of helper T lymphocyte; pCTL, precursor of cytotoxic T lymphocyte.

critical role in protecting cells against the adverse effects of heat shock and other stresses (reviewed in 44, 45). In addition, heat shock proteins appear to play an important role in the function of cells in the immune system (reviewed in 46, 47). Recently, our laboratory reported a study on the effect of dietary restriction and aging on the induction of heat shock protein 70 (hsp70) expression by hyperthermia and IL-2 expression by ConA. We found that the induction of hsp70 expression (protein and mRNA levels) and IL-2 expression (activity and mRNA levels) decreased significantly with age. The decrease in IL-2 mRNA level with age was attenuated by dietary restriction. However, dietary restriction had no significant effect on the age-related decrease in hsp70 expression (48). Thus, our study indicated that the effect of dietary restriction in altering the levels of mRNA transcript appears to be gene-dependent.

How does dietary restriction specifically alter the transcription of a gene? It is currently known that transcription requires the recognition of numerous DNA sequences by a diverse group of proteins, which are termed transcription factors. Transcription factors represent one of the largest and most diverse classes of DNA-binding proteins, and they regulate gene expression at the level of transcription. Over the past decade, it has become evident that these proteins play a critical role in development, differentiation and cellular proliferation (68-70). Thus, changes in the activities or levels of transcription factors could be a mechanism whereby dietary restriction alters the transcription of genes in a specific manner. We postulated that dietary restriction alters a transcription factor that plays a critical role in the regulation of the IL-2 gene. Transcription of the IL-2 gene is regulated by the binding of several transcription factors (NFAT, NF-κB, AP-1, AP-3, and OCT), of which NFAT (nuclear factor of activated T cell) plays a predominant role (71,72). Using nuclear extracts from ConA-stimulated T cells isolated from young and old rats

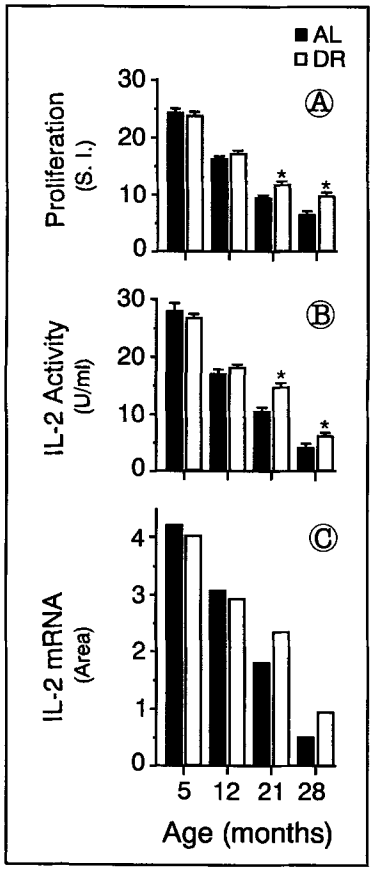

Figure 1. Influence of dietary restriction and aging on mitogen-induced lymphocyte proliferation and IL-2 expression in F344 rats. Spleen lymphocytes were isolated from dietary restricted (DR) and ad libitum (AL) fed rats of various ages and were cultured with or without ConA. Proliferation was measured by liquid scintillation counting and expressed as stimulation index (S.I.), which is defined as the ratio of [^{3}H]-thymidine incorporation by ConA divided by [^{3}H]-thymidine incorporation without ConA. IL-2 activity in supernatant of cultured lymphocytes was measured using an IL-2-dependent T cell line and was expressed as Unit/ml. IL-2 mRNA levels were determined by dot blot hybridization using cytoplasmic extracts prepared from lymphocytes pooled from the 4 animals. The blot was analyzed by densitometry and expressed as arbitrary units (area). Data were taken from Pahlavani, et al. (40). The values (*) for the dietary restricted rats were significantly different from the values for the rats fed ad libitum at $p<0.05$.

fed *ad libitum* and old rats fed a restricted diet, our laboratory measured the induction of NFAT binding activity with a gel shift assay (56). The results of this study which is summarized in Figure 2 show that the induction of NFAT binding activity was significantly less in nuclear extracts from T cells isolated from old rats compared to the level from young rats. The binding activity of NFAT was significantly higher in nuclear extracts isolated from old rats fed a restricted diet than old rats fed ad libitum. In addition, the data in Figure 2 show that the increase in NFAT binding activity in dietary restricted rats correlates with an increase in IL-2 gene expression. Thus, our study seems to indicate that dietary restriction alters the transcription of IL-2 through changes in the NFAT transcription factor.

Immunomodulatory Role of Dehydroepiandrosterone

Research over the past decade has shown that the androgenic hormone dehydroepiandrosterone (DHEA) produces diverse biological effects in humans and experimental animals. For example, DHEA has been shown to be involved in various physiological/pathological conditions such as obesity, cancer, diabetogenesis, atherosclerosis, osteoporosis, etc. (for review see ref 31,32,73). In 1979, Schwartz (74) suggested that DHEA may *mimic the* beneficial effects of dietary restriction, in modulating various age-associated diseases, e.g., cancer. The mechanisms by which DHEA exerts its diverse action are unknown. However, DHEA is of interest to gerontologists because the adrenal production of DHEA decreases dramatically with increasing age in mammals and there is an inverse relationship between plasma concentration of DHEA and incidence of various age-associated diseases, such as infection and cancer. Moreover, DHEA therapy has been shown to reduce the occurrence of such diseases (75-77).

DHEA does not appear to have any effect on the histomorphology of lymphoid tissues or the immune function of the uninfected host. However, in the presence of pathogenic infection, DHEA exhibits an immunoprotective action on the host. For example, a single subcutaneous injection of DHEA into mice resulted in protection from lethality of various infectious agents such as Coxsackie B enterovirus, herpes type 2, and *Enterococcus faecalis* (78,79). Similarly, DHEA therapy reduced mortality in mice infected with West Nile virus (WNV), Sinbis virus neurovirulent, and Semliki Forest virus (80). Subcutaneous injection of DHEA prevented the development of WNV lethality in cold stressed mice (80). In another study, it was reported that DHEA therapy enhanced the immunologic response of rats, which were immunosuppressed by dexamethasone treatment and then infected with *Cryptosporidium parvum* (81). The levels of proinflammatory cytokine, i.e., interleukin-6, were found to be lower in DHEA-treated animals that were exposed to various stressors such as sepsis, endotoxin exposure, or burns (82). These studies did not provide definitive evidence that the antibacterial and/or antiviral effect of DHEA as a result of a direct effect of the steroid on pathogens; however, they indicated that protection from lethality of various infectious agents was due to the stimulatory effect of DHEA on the immune system.

During the last several years, a number of studies have been reported on the effect of DHEA on various immune responses. These studies, which were conducted mostly in young mice (2 to 7 months) are listed in Table 2. In these studies, DHEA or DHEAS (dehydroepiandrosterone-sulfate) was supplemented in the diet, in drinking water, or administered subcutaneously. The DHEA concentrations ranged from 10 µg to 10 mg/day,

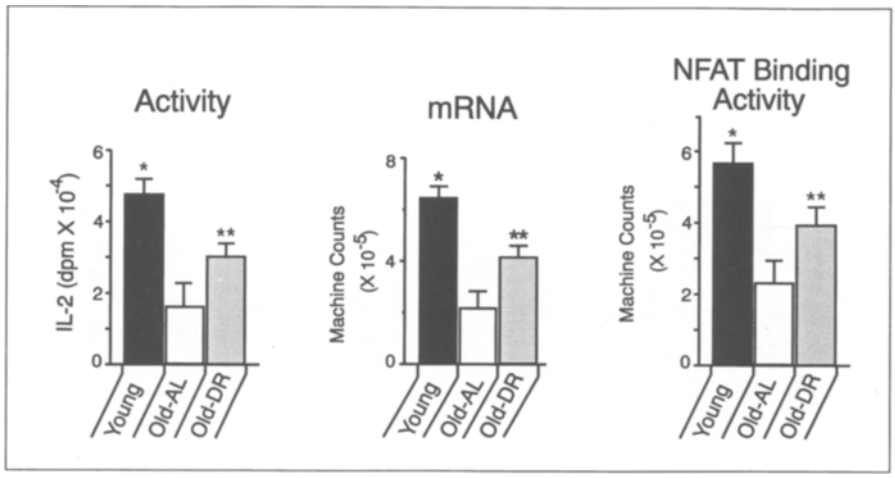

Figure 2. Effect of age and dietary restriction on the induction of IL-2 activity, IL-2 mRNA levels, and DNA binding activity of transcription factor NFAT. Splenic T cells isolated from control young (6 month) and old (24 month) rats and dietary restricted old (24 months) rats were stimulated with ConA. IL-2 activity was measured using an IL-2-dependent T cell line (CTLL-20) and mRNA levels were determined by Northern blot analysis. The NFAT binding activity of the nuclear extracts was measured by the gel mobility shift assay. Data were taken from Pahlavani, et al. (66). The value (*) for young rats was significantly different from the value for the old rats fed ad libitum and dietary restricted old rats at $p<0.001$. The values (**) for dietary restricted old rats were significantly different from the value for the old rats fed ad libitum at $p<0.05$.

and the duration of treatment ranged from 4 days to 18 months. Following the treatment, lymphocytes from various lymphoid tissues (e.g., spleen, thymus, bone marrow, or blood) were used to assess the effects of DHEA/S on cellular and/or humoral immune responses. Although the majority of the studies were conducted in vivo, in several in vitro studies, the splenocytes or thymocytes were directly exposed to various concentrations of DHEA/S ranging from 10^{-5} to 10^{-11}M for duration of 45 min to 48 hours. It is clear from the data in Table 2 that the effects of DHEA/S vary considerably from one study to another. For example, Daynes et al. (83) reported that exposure of mice splenic T cells to DHEA/S, prior to mitogenic stimulation, produces a high level of cytokines (i.e., IL-2 and IFN-γ) compared to the splenic T cells isolated from the non-treated mice. However, a study from Padgett et al. (84) showed that exposure of mice lymphocytes to DHEA resulted in a decrease in various immune parameters such as mitogenesis and cytokine (e.g., IL-2 and IL-3) production. In addition, a study from Risdon et al. (85) showed that DHEA treatment (0.45% DHEA in diet for a period of 6 to 8 weeks) in mice had no significant effect on various immune parameters including antibody responses, cutaneous sensitivity reactions, NK cell activity, or graft-versus-host reactions.

In 1991, Daynes et al. (16) suggested that some of the age-related decline in the immune system (i.e., deficit in T cell function) is linked to the decline in DHEA production that occurs during the aging process. They provided evidence showing that chronic oral administration of DHEAS in mice reverses immunosenescence and maintains their capacity to produce a "young-like" pattern of immunologic response, a response that diminishes markedly in untreated old mice. For example, the quantity and pattern of cytokines (i.e., IL-2, IL-3, GM-CSF, IL-4, IL-5, and IFN-γ) produced by the activated T cells, and the antibody response to a foreign antigen obtained from the DHEA-treated old mice were similar to the non-treated young mice. More astonishingly, they reported that even acute treatment, either giving a single injection of DHEA or atopic application of DHEA to the skin of old mice 24 hours prior to assay or immunization was as effective as chronic oral administration (16). Subsequent studies from Daynes laboratory have shown that DHEA treatment reverses age-associated changes in humoral immune responses in mice. For example, they reported that treatment of aged mice with DHEA prior to immunization with a recombinant vaccine against hepatitis B antigen (rHBsAg) enhanced their capacity to elicit a vigorous primary and secondary antibody response against hepatitis B viral antigen (17). These studies strongly suggested an anti-immunosenescence role of DHEA and supported a "cause-effect" relationship between the decrease in endogenous production of DHEA/S during aging and the loss of immunologic responses with age. Although Daynes et al. (83) reported that in vitro DHEA treatment increases IL-2 and IFN-γ production in a mouse model, other studies found no changes in immune responses when lymphocytes were exposed directly to DHEA using mice or rat models (84-86,87,88). For instance, in a recent study in our laboratory, we tested a broad range of DHEA concentrations (i.e., from 10^{-8} up to 10^{-1} μg/ml) and found that the concentration of 10^{-6} to 10^{-4} μg/ml only slightly increased mitogen-induced lymphocyte proliferation in young and old rats. In this study, we observed that regardless of whether lymphocytes were cultured in FCS (fetal calf serum) or with serum-free medium (Nutridoma-SR), the *in vitro* DHEA treatment had no significant effect on the

Table 2. Immunomodulatory Role of DHEA

Species	Strain	Age (months)	Lymphoid (Cells)	Immune Parameter	DHEA/S Dose	DHEA/S Treatment	Change with DHEA	Ref
Mouse	NZB/WF1	7	Blood/Serum	IgG	0.4%	in diet, 5 mo	No change	89
				IgM			No change	
			Macrophage	Phagocytosis			No change	
			Splenocyte	Proliferation (ConA)			No change	
				Proliferation (PHA)			No change	
				Proliferation (LPS)			No change	
	C3H/HeN	1	Splenocyte	Proliferation (ConA)	10^{-5} - 10^{-8} M	in vitro, 24 h	No change	87
				Proliferation (PHA)			No change	
				Proliferation (PWM)			No change	
				Proliferation (LPS)			No change	
	C57BL/6N	2	Splenic T cell	CD4+ T cell (%)	10 µg/day	S.C., 3 wk	Increase	80
				CD8+ T cell (%)			Increase	
				B cells (%)			Increase	
	C57BL/6J	2	Splenocyte	Antibody (SRBC)	0.4%	in diet, 2 mo	No change	90
				NK activity			No change	
				C.S.R.			No change	
				G.V.H.			No change	
	C3H/HeN	5-25	Splenic T cell	IL-2	100 µg/ml	oral, 11 mo	Increase	17
				IL-4			Decrease	
				IL-5			Decrease	
				IFN-γ			Decrease	
	C57BL/6J	2	Splenocyte	Proliferation (ConA)	$5X10^{-6}$ - 10^{-9} M	in vitro, 24 h	Decrease	84
				IL-2 and IL-3			Decrease	
	B6D2F1	2	Splenocyte (Irradiation)	Erythropoiesis	0.4%	in diet, 2 wk	No change	88
				Thymocyte No.			No change	
				NK activity			No change	
			Bone Marrow (Irradiation)	B cells (B220+)			Decrease	
	CB-17	5	Splenocyte	Antibody (pnu vaccine)	10 µg/d	S.C., 1 wk	Increase	92
		17		Antibody (pnu vaccine)			Increase	
	Balb/c	5	Splenocyte	Antibody (pnu vaccine)	100 µg/d	S.C., 1 wk	Increase	92
		24		Antibody (pnu vaccine)			Increase	
	C57BL10.SN	4.5	Splenocyte	Proliferation (PHA)	0.4%	in diet, 3.5 mo	Increase	93
				Proliferation (ConA)			Increase	
				NK activity			No change	
	C3H/HeN	5-25	Splenic T cell	IL-2	100 µg	S.C., 24 h	Increase	94
				IL-4			Decrease	
				IFN-γ			Decrease	
	C3H/HeN	6-28	Splenic T cell	IL-6	100 µg/ml	oral, 2.5 mo	Decrease	95
			Blood/Serum	IgM			Decrease	
				IgA			Decrease	
				IgG1			Decrease	
	(C3HXC57)F1	5	Splenocyte	IL-2	10^{-7} mole/liter	in vitro, 45 min	Increase	91
				IFN-γ			Increase	
	(C3HXC5B)F1	10-25	Splenocyte	Antibody (rHBsAg)	100 µg	S.C., 24 h	Increase	17
	Balb/c	6	Splenic T cell	IL-2	100 µg/d	S.C., 3 d.	Increase	77
				IL-4			No change	
	Balb/c	6-24	Splenic T cell	IL-6	100 µg/ml	oral, 2.5 mo	Decrease	95
	Balb/c	3-28	Splenocyte	IL-2	100 µg/ml	oral, 18 mol	Increase	16
				IL-3			Increase	
				GM-CSF			Increase	
				IL-4			Decrease	
				IL-5			Decrease	
				IFN-γ			Decrease	
				Antibody (OVA)			Increase	
	C57BL/6J	2	Splenocyte	Antibody (CVB4)	1 g/kg	S.C., 10 d.	No change	78

(Continued on next page)

Table 2 *(continued)*

Species	Strain	Age (months)	Lymphoid (Cells)	Immune Parameter	DHEA/S Dose	DHEA/S Treatment	Change with DHEA	Ref
Mouse	Balb/c	2	Thymus	Involution by (Dex. treated)	1.6 mg/ml	S.C., 4 d.	Decrease	96
			Thymocyte	Cell Lysis (Dex. treated)		(in vitro)	No change	
	C57BL/6J	2	Splenocyte	Proliferation (ConA)	10^{-6} - 10^{-9} M	in vitro, 24 h	Decrease	83
				Proliferation (LPS)			Decrease	
				IL-2 activity			Decrease	
				IL-3 activity			Decrease	
	HRS/J	2	Splenocyte	Antibody (CVB4)	1 g/kg	S.C., 10 d.	No change	78
Rat	S. Dawley	2	Splenocytes	Proliferation (ConA)	120 mg/day	S.C., 2 wk	No change	80
				Proliferation (LPS)			No change	
				NK cell activity			No change	
				Antibody (SRBC)			No change	
	F344	6	Splenocyte/T cell	Proliferation (ConA)	10^{-1}-10^{-8} mg/ml	in vitro, 24 h	No change	86
				IL-2 activity			No change	
				IFN-γ			No change	
		24	Splenocyte/T cell	Proliferation (ConA)	10^{-1}-10^{-8} mg/ml	in vitro, 24 h	No change	
				IL-2 activity			No change	
				IFN-γ			No change	
Human	HIV patient	>18	Blood (MNC)	CD4+ T cells (%)	750 mg/day	oral, 4 mo	No change	97
				CD48 T cells (%)			No change	
				Serum p24 Antigen			No change	
	HIV patient	——	ACH-2 cell line	NF-κB binding	100 μM	in vitro, 12 h	Decrease	98
	Healthy	?	Blood	IL-2	10^{-8} - 10^{-11} M	in vitro, 48 h	Increase	99
				IL-2 mRNA			Increase	
				Cytotoxicity			Increase	
	Healthy	53-69	Blood MNC	Proliferation (PHA)	50 mg	oral, 20 wk	Increase	100
				IL-2 activity			Increase	
				IL-2R			Increase	
				NK activity			Increase	
	Cell line	——	——————	Antibody (CVB4)	2-20 μM	in vitro, 12 h	No change	78

MNC, mononuclear cell; ConA, concanavalin A; PHA, phytohemagglutinin; LPS, lipopolysaccharide; PWM, pokeweed mitogen; IL-2, interleukin-2; IL-4, interleukin-4; IL-5, interleukin-5; IL-6, interleukin-6; IFN-γ, interferon-gamma; G-M-CSF,granulocyte-macrophage-colony stimulating factor; TNF-α, tumor necrosis factor-alpha; SRBC, sheep red blood cell; OVA, ovabumin; NK, natural killer; C.S.R., cutaneous sensitivity reaction; G.V.H., graft versus host; Dex, dexamethasone; rHBsAg., recombinant hepatitis B antigen; S.C., subcutaneous.

proliferative response to ConA or IL-2 or IFN-γ production in lymphocytes isolated from either young or old rats (86). Thus, while some studies in mice have shown that DHEA has immunostimulatory and anti-immunosenescenct effects, these results have not been consistently reported by others. Further studies are needed to confirm the immunoenhancing effect of DHEA in experimental animals and humans.

Immunomodulatory Effect of Melatonin

The pineal hormone melatonin (MLT) which is principally involved in photoperiodic and seasonal information has also been linked to modulating the immune function (reviewed in ref 18,101-103). The initial studies on the immunomodulatory role of MLT were reported by Maestroni et al. (104,105) who showed that the inhibition of MLT synthesis by β-blockers or permanent lighting suppressed the immune function in mice and that MLT therapy counteracts these effects. Subsequent studies from Maestroni's group and others have indicated that MLT has an immunomodulatory effect, i.e., it enhances the cellular and humoral immune responses in mice (reviewed in 102). These studies are summarized in Table 3. In these studies, MLT was administered subcutaneously to pinealectomized and/or non-pinealectomized young mice (2 to 8 months). The concentrations used ranged from 1.5 pg/kg to 10 mg/kg for duration ranging from 3 days to 10 months. In a few in vitro studies, the splenocytes or thymocytes were directly treated with various concentrations of MLT (ranging from 1.5 pg/ml to 1.5 mg/ml) for duration of 25 min to 24 hours. After MLT treatment, splenocytes and/or thymocytes were isolated and used to assess the effect of MLT on cellular and humoral immune responses. As shown in Table 3, pinealectomy reduces various immunologic functions, e.g., IL-2 production, NK cell activity,

Table 3. Immunomodulatory Role of Melatonin

Species	Strain	Age (weeks)	Lymphoid (Cells)	Immune Parameter	Pinealectomy Effect	Melatonin Dose	Change with Melatonin	Ref
Mouse	C57BL/6J	10	Splenocyte	Antibody Response	Decrease	10 mg/kg, S.C., 4 d	Increase	104
	C57BL/6J	8	Splenocyte	Proliferation (ConA)	Decrease	10 mg/kg, S.C., 4 d	———	107
	C57BL/6J	8	Bone Marrow	G/M-CFU	Decrease		———	
	Balb/c	10	Bone Marrow	Granulocytes	Decrease		Increase	
				Macrophages	Decrease		Increase	
	Balb/c	5	Splenocyte	Proliferation (ConA)	Decrease	10 mg/kg, S.C., 4 d	———	108
	Balb/c	5	Splenocyte	Cytotoxicity	Decrease	0.1 mg/kg, S.C., 16 h	Increase	109
	C57BL/6J	5	Splenocyte	IL-2 activity	Decrease	100 mg/kg, S.C., 16 h	Increase	110
				NK activity	Decrease		Increase	
	Balb/c	3	Splenocyte	Antibody (SRBC)	———	10 mg/kg, S.C., 1 wk	Increase	105
				Proliferation (PHA)	———		Increase	
				Proliferation (LPS)	———		Increase	
				Proliferation (MLR)	———		Increase	
	C57BL/6J	2	Splenocyte	Proliferation (ConA)	———	10 mg/kg, S.C., 4 d	Increase	111
				NK activity	———		Increase	
				TNF-α	———		Increase	
	(C57BL/10 X BDA/2)F1	2	Splenocyte	Antibody (HRB)	———	10 mg/kg, S.C., 4 d	Increase	19
				Helper T cell activity	———		No change	
				IL-2 activity	———		No change	
	Balb/c	60	Splenocyte	DTH (Oxazalone)	———	10 µg/ml, 10 mo	Increase	115
	C57BL/6	76	Splenocyte	DTH (Oxazalone)		10 µg/ml, 7 mo	Increase	
	(C57BL/10 X BDA/2)F1	12-19	Splenocyte	Antibody (HRB)	———	10 mg/kg, S.C., 4 d	Increase	106
				Helper T cell activity	———		Increase	
				IL-2	———		Increase	
	C57BL/6J	2	Splenocyte	G/M-CFU	———	10 mg/kg, S.C., 4 d.	Increase	112
	Balb/c	12-72	Thymocyte	Weight	———	10 µg/ml, 4 mo	Increase	113
			Splenocyte	Weight	———		No change	
				Cell number			Increase	
				CD4+ T cells			No change	
				Proliferation (PHA)			Increase	
				Proliferation (ConA)			Increase	
	Balb/c	24-40	Serum	Viremia (SFV)	———	500 µg/kg, S.C., 3 d	Decrease	114
	Balb/c	8-72	Thymus/spleen	Apoptosis by Dex.	———	1 pg -1µg/ml, 20 h	No change	116
	C57BL/6J	2	Splenocyte	IL-4	———	1 mg/kg, S.C., 3 wk	Increase	117
				Antibody Response	———		Increase	
	C57BL/6J	3	Splenocyte	Antibody (SRBC)	———	10 mg/kg, S.C. 1 wk	Increase	105
				Proliferation (MLR)	———		Increase	
	House mouse	5	Thymus	Involution by Dex.	Increase	10 mg/kg, S.C., 4 d	Decrease	118
	C57BL/6J	8	Splenocyte	Proliferation (ConA)	———	20 µg/kg, S.C., 4 d	Increase	119
				Antibody (SRBC)	———		Increase	
			Thymus	Involution	———		Decrease	
	(C57XDBA)F1	2	Splenocyte	TNF-α	———	10 mg/kg, S.C., 5 d	Increase	120
				MHC-II	———		Increase	
				IL-1	———		Increase	
Rat	Wistar	4	Thymocyte	Apoptosis by Dex.	———	10^{-9} -10^{-11} M, 6 h	No change	121
	F344	24	Splenocyte/T cell	Proliferation (ConA)	———	10 pg-10 mg/ml, 24 h	No change	122
				IL-2 activity			No change	
				IFN-γ			No change	
		96	Splenocyte/T cell	Proliferation (ConA)	———	10 pg -10 mg/ml, 24 h	No change	122
				IL-2 activity			No change	
				IFN-γ			No change	

(Continued on next page)

Table 3 *(continued)*

Species	Strain	Age (weeks)	Lymphoid (Cells)	Immune Parameter	Pinealectomy Effect	Melatonin Dose	Change with Melatonin	Ref
Hamster								
	Syrian	?	Splenocyte	Antibody Response		1 mg/kg, S.C., 7 wk	Increase	123
Human								
		??	Blood/serum	sIL-2R TNF	———	1-10 pg/ml, S.C., 6 d	No change No change	125
		30-50	Blood (MNC) (irradiation)	Chromosome Aberration	———	0.5-2μM, 20 min	Decrease	126
		35-55	Blood (MNC)	IL-1mRNA	———	5 X 10^{-11} M, 12 h	Increase	124

MNC, mononuclear cell; ConA, concanavalin A; PHA, phytohemagglutinin; MLR, mixed lymphocyte reaction; LPS, lipopolysaccharide; PWM, pokeweed mitogen; IL-1, interleukin-1; IL-2, interleukin-2; sIL-2R, soluble IL-2 receptor; IL-4, interleukin-4; IFN-γ, interferon-gamma; G/M-CFU, granulocyte/macrophage-colony forming unit; TNF-α, tumor necrosis factor; SRBC, sheep red blood cell; HRB, horse red blood; NK, natural killer; MHC, major histocompatibility complex; DTH, delayed type hypersensitivity; Dex, dexamethasone; S.C., subcutaneous.

antibody response, and the number of bone marrow progenitor cells (granulocyte macrophage-colony forming unit), and MLT treatment counteracts the immunosuppression caused by acute restraint stress, corticosterone treatment, or pinealectomy). For example, studies by Caroleo et al. (19,106) showed that MLT administration reversed thymus involution and restored the proliferative response of thymocytes to mitogenic lectins in non-pinealectomized old mice, and in the young mice that were immunodepressed by acute restraint stress or by corticosterone treatment (101).

MLT administration has been shown to have beneficial effects not only on histomorphology of the lymphoid tissues (e.g., thymus and spleen), but also on lymphoid cells (e.g., T cells and B cells). For example, treatment of both normal or immunosuppressed mice with MLT resulted in an increase in the function of B cells in producing antibodies *in vitro* as well as *in vivo* (34). Likewise, the production of various cytokines (e.g., IL-2 and IFN-γ) by spleen lymphocytes increases with MLT treatment in non-pinealectomized mice (Table 3). MLT therapy also has stimulating effects on the function of macrophages. Antigen presentation by splenic macrophages to T cells was shown to increase in mice treated with MLT. The enhancement in macrophage function was shown to be correlated with an increase in the expression of the major histocompatibility complex, class II molecules, as well as an increase in IL-1 and TNF-α production (120). Moreover, the expression of IL-1 mRNA levels by monocytes was found to increase in humans treated with MLT (124). Although a number of *in vivo* studies indicate that MLT therapy enhances the immunologic function, the studies conducted *in vitro* show that MLT treatment has no effect on the function of lymphocytes in rodents, raising the possibility that the effects of MLT *in vivo* are indirect. For example, our laboratory has recently reported that treatment of mixed spleen lymphocyte populations or purified T cells with MLT did not significantly alter ConA-induced lymphocyte proliferation or IL-2 or IFN-γ expression in cells isolated from either young or old rats (122). Similarly, a study by Provinciali et al. (116) in mice has also shown that in vitro MLT treatment (ranging from 1.5 pg/μl to 1.5 mg/ml) produced no effect on the percent apoptotic nuclei induced by serum deprivation or by dexamethasone treatment in thymocytes or splenocytes. In addition, a study by Karasek et al. (127) showed that *in vitro* MLT treatment (at concentrations of 10^{-5} M to 10^{-11} M) was ineffective on proliferation of transplanted hepatoma cells into the Syrian hamster. One possible explanation for the lack of effect of MLT *in vitro* could be due to the indirect action of this hormone. It is highly probable that the immunostimulatory effect of MLT that has been reported *in vivo* could result from an indirect effect that might involve other hormones or cytokines. Therefore, it remains to be determined whether the immunostimulatory and anti-immunosenescent effect of MLT are restricted only to in vivo and/or to *in vitro* studies.

Recent studies suggest that MLT may have therapeutic potential in the treatment of immunosuppressive conditions such as cancer and aging. The immunostimulatory effects of MLT are of particular interest to aging because the circulatory level of MLT decreases with age in humans and rodents (18,32,115). In addition, several clinical and experimental studies suggest that the plasma level of MLT is inversely correlated with the occurrence of neoplastic diseases (103,128-134). In experimental animals, the removal of MLT by pinealectomy was shown to stimulate the growth of a variety of tumors (e.g., fibrosarcomas, ovarian carcinomas, carcinogen-induced breast tumors, and melanomas) (129-132). Hence, the observation that neoplasia is associated with low circulatory levels of MLT has offered a rationale for a therapeutic intervention, and a number of recent studies have demonstrated that MLT therapy suppresses cancer growth (reviewed in ref 133,135). Several clinical studies have shown that MLT therapy, alone or in combination with IL-2, significantly decreases the progression of various tumors, e.g., carcinomas (136), melanoma (135), renal (133,135), lung (135-138), and breast tumors (139-141). Cancer patients, who were unable to tolerate the IL-2 therapy alone and who did not respond to standard anti-cancer chemotherapies, were shown to have increased IL-2 tolerance,

and they exhibited an effective antitumor defense response when they were treated with a combination of MLT and IL-2 (134,135,142-148). Although the exact mechanism responsible for the anti-neoplastic action of MLT has not been determined, it has been suggested that MLT mediates its effect on hematopoietic progenitor cells, i.e., it increases the number of the granulocyte macrophage-colony forming unit (34,117). Additionally, MLT enhances IL-2-induced eosinophilia in cancer patients by a mechanism that involves the synthesis of other T cell cytokines such as IL-4 and IL-5 (117). Another proposed mechanism is that MLT may exert its anti-cancer effect by enhancing immune function, i.e., by increasing the activity of tumor killing cells, such as NK cells and lymphokine-activated killer cells (134,135). Thus these studies indicate that MLT therapy reduces tumor growth and a combination of MLT and IL-2 is a well-tolerated therapeutic strategy in treating patients with cancer.

In recent years, a great deal of interest has been generated over the possibility that MLT substitution therapy might also reverse the age-related changes in immunity. Three preliminary reports indicate that MLT therapy reverses immunosenescence in mice. Mocchegiani et al. (113) reported that MLT treatment in old mice increased the proliferative response of lymphocytes to mitogen to a level that is similar to young mice. Also, a study by Caroleo et al. (19) showed that MLT administration increased the induction of IL-2 expression by ConA in old mice to a level that is similar to young mice. Pierpaoli and Regelson (115) recently reported that survival of old mice was increased when the pineal glands of young mice were grafted into old mice. Furthermore, they showed that MLT administration prevented the age-associated thymic involution, and restored the cell-mediated delayed-type hypersensitivity response (115). In this study, the anti-aging and anti-immunosenescence effect of pineal transplant was attributed to the up-regulation of MLT secretion in the old mice that received the graft. However, this report has been subjected to criticism because the pineal gland of the strain of mice used in this study does not produce MLT (144). Thus, based on these limited studies, it is difficult at present to draw any conclusions on the efficacy of MLT in reversing/restoring aging immune system.

Influence of Exercise on Immune Function

Clinical studies have clearly demonstrated that regular exercise can lead to a significant improvement of cardiopulmonary function, and it is believed that good physical conditioning is equated with an improved resistance to infections, presumably by enhancing the immune function (150). In recent years, limited attention has been given to the role of exercise on the immune function and the available studies are presented in Table 4. The research on the effects of exercise on immune function has primarily been conducted in young subjects; and there are only a few studies available on the effects of exercise on the aging immune system. Some reports indicated that exercise resulted in an increase in the immune function, and others reported that exercise induces immunosuppression. The discrepancy of the results could presumably arise from a number of factors such as experimental design, the intensity and duration of the imposed exercise and the age and heterogeneity of the groups of subjects investigated. In general, regular moderate exercise has been shown to increase cellular and humoral immune responses (151, 155, 171, 172). For example, Tharp and Preuss (155) showed that mitogen-induced lymphocyte proliferation was enhanced in rats subjected to treadmill exercise for 8 weeks compared to the sedentary controls. Similarly, Mahan et al. (151) reported that mitogen-induced lymphocyte proliferation was increased when rats underwent moderate swimming exercise for 10 weeks compared to the sedentary controls. In contrast to the immunoenhancing effect of moderate exercise, strenuous exercise has been associated with immunosuppression. For example, mitogen-induced lymphocyte proliferation (151,166,173-175) and IL-2 production (22,23,167) have been reported to decrease with strenuous exercise. The suppression of the immune function is correlated with the intensity of exercise, the greater the intensity, the

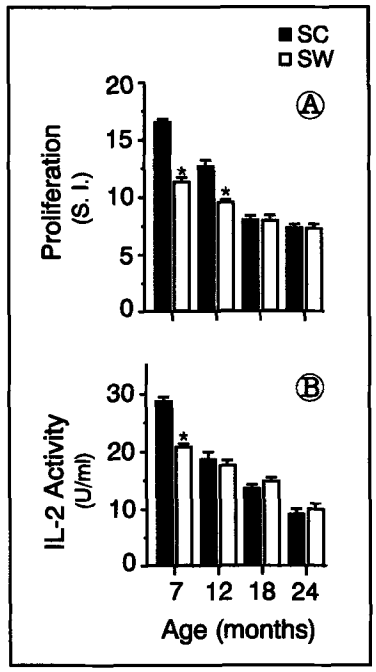

Figure 3. Effect of exercise and aging on mitogen-induced lymphocyte proliferation and IL-2 expression in F344 rats. Spleen lymphocytes were isolated from swim-exercised (SW) and sedentary control (SC) rats of various ages and were stimulated with ConA. Proliferation was measured by scintillation counting and expressed as stimulation index (S.I.). IL-2 activity in the supernatant of cultured lymphocytes was measured using an IL-2-dependent cell line and was expressed as Unit/ml. Data were taken from Pahlavani, et al. (22). The values (*) for the swim-exercised rats were significantly different from the values for the sedentary control rats at $p<0.001$.

Table 4. Effect of Exercise on Immune Function

Species	Strain	Age (months)	Lymphoid Cells	Immune Parameter	Exercise Protocol	Exercise Duration	Change with Exercise	Ref
Rats								
	Wistar	3	Splenocyte	Proliferation (ConA)	Swimming	10 wk	Decrease	151
	F344	8	Splenocyte	Proliferation (ConA) IL-2 activity Cytotoxicity	Treadmill	15 wk	Decrease Decrease Decrease	23
		17	Splenocyte	Proliferation (ConA) IL-2 activity Cytotoxicity	Treadmill	15 wk	Decrease Decrease Decrease	
		27	Splenocyte	Proliferation (ConA) IL-2 activity Cytotoxicity	Treadmill	15 wk	Increase Decrease Decrease	
	S. Dawley	4	Blood (MNC)	Proliferation (ConA) IL-2 activity NK activity CD4+ T cell (%) CD8+ T cell (%)	Swimming	5 d.	Increase Increase Increase Decrease Decrease	152
	Wistar	3	Splenocyte	Proliferation (PHA)	Treadmill	2 wk	Decrease	153
	SHR	6	Thymocyte Splenocyte	Proliferation (ConA) Proliferation (ConA)	Treadmill	4 wk	Increase increase	154
	S. Dawley	4	Blood (MNC)	Proliferation (ConA)	Treadmill	8 wk	Increase	155
	F344	5	Splenocyte	Proliferation (ConA) Proliferation (LPS) IL-2 activity	Swimming	6 mo	Decrease	22
		12	Splenocyte	Proliferation (ConA) Proliferation (LPS) IL-2 activity	Swimming	6 mo	Decrease	
		22	Splenocyte	Proliferation (ConA) Proliferation (LPS) IL-2 activity	Swimming	6 mo	Decrease	
Mouse								
	C3He	3	Splenocyte	NK activity	Treadmill	10 wk	Increase	156
	Balb/c	3 14	Macrophage Macrophage	Phagocytosis Phagocytosis	Swimming Swimming	4 wk 4 wk	Increase Increase	157
	C57BL	3	Splenocyte	Proliferation (ConA) Proliferation (PHA) Proliferation (LPS) Antibody (SRBC)	Treadmill	3 wk	No change No change Increase No change	158
	Balb/C	4 15	Splenocyte	Proliferation (PHA) Proliferation (PHA)	Swimming Swimming	3 wk 3 wk	Decrease Decrease	159
	C57BL/6J	3	Splenocyte	Proliferation (ConA)	Treadmill	6 wk	Decrease	160
Hamster								
	Golden	2	Blood	Fever (LPS) IL-6	Run. Wheels	3 wk	No change No change	161
Human								
		19-27	Saliva	IgA	Ski athletes	50 km	Decrease	162
		71-73	Blood	NK activity	Aerobic Train.	16 wk	Increase	163
		?	Blood	Proliferation (PHA) Proliferation (PWM)	Water Polo	———	Decrease Decrease	164
		24-26	Blood (MNC)	Proliferation (ConA)	Ergometer	1 wk	Decrease	165
		25-50	Blood	Proliferation (PHA) Proliferation (ConA) Proliferation (PPD)	Marathon Run	42km	Decrease Decrease Decrease	166
		20-22	Blood (MNC)	NK activity IL-2	Cyclists	20,000 Km	Increase Decrease	167
		21-39 >65	Blood (MNC) Blood (MNC)	NK activity NK activity	Ergometer Ergometer		Increase Increase	168

(Continued on next page)

Table 4 *(continued)*

Species	Strain	Age (months)	Lymphoid Cells	Immune Parameter	Exercise Protocol	Exercise Duration	Change with Exercise	Ref u
Human (continued)								
		27-40	Blood	Lymphocyte number	Cyclists	10 miles/d	Increase	169
		27-40	Blood	IgG IgA IgM Cytotoxicity	Runners	10 miles/d	No change No change No change No change	170

MNC, mononuclear cell; ConA, concanavalin A; PHA, phytohemagglutinin; LPS, lipopolysaccharide; PWM, pokeweed mitogen; PPD, purified protein derivative; IL-2, interleukin-2; IL-6, interleukin-6; SRBC, sheep red blood cell; NK, natural killer.

higher the immunosuppression. Several investigators have suggested that the immunosuppressive effect of strenuous exercise may be indirectly related to changes in hormones associated with the stress response, i.e., glucocorticoids (176-178). Thus, a vigorous and exhaustive exercise program may be a stressor that leads to the elevation of blood glucocorticoid levels resulting in a suppressive effect on the immune system.

Because aging is often associated with an increase in the incidence and occurrence of infectious diseases and because of the common belief that exercise training increases resistance to infection, it would be of interest to know to what extent exercise can modulate the aging immune system. In 1988, our laboratory reported that exercise resulted in suppression of immune function in Fischer 344 rats (22). In this study, rats from four different age groups (1, 6, 12, and 18 months, initially) were subjected to a swim-training program for a period of 6 months. The sedentary control groups were maintained in a shallow water for the same period of time as the swim-exercise groups. Lymphocyte proliferation and IL-2 production in isolated splenocytes were measured. The proliferative response of lymphocytes to ConA in both swim-exercised and sedentary control rats declined significantly with increasing age. We found that swimming exercise program resulted in a significant reduction in lymphocyte proliferation in 7- and 12-month rats when compared to their age-matched sedentary control groups. No significant differences in mitogenesis were observed between exercised and sedentary control rats from the 18- and 24- month age groups. Similarly, the induction of IL-2 production declined significantly with age in both the swim-exercised and sedentary control rats. For the 7-month-old rats only, the swimming-exercised was associated with significantly lower levels of IL-2 production when compared with sedentary controls. Subsequently, reports from other laboratories using different exercise protocols confirmed our finding that strenuous exercise suppresses immunologic function. For example, using the same strain of rats, Nasrullah and Mazzeo (23) reported the effect of 15 weeks of endurance training by treadmill running on T cell proliferation, IL-2 production and cytotoxicity in 8, 17, and 27-month-old rats. They found that there was a significant age-related decline in T cell proliferation for both exercised and non-exercised/sedentary rats. Treadmill exercise appeared to significantly diminish T cell proliferation in the young and middle-aged exercised rats when compared with their sedentary controls, similar to the findings reported earlier by our laboratory (22). In our study, we did not observed significant difference in mitogen-induced proliferation or IL-2 production between the swimming exercise and non-exercised old rats. However, Nasrullah and Mazzeo (23) reported that 15 weeks of endurance training improved mitogenesis and IL-2 production in older animals.

Several studies in humans have also shown that exercise results in suppression of certain immune responses. For example, Papa et al. (164) showed significant decline in the response of peripheral blood lymphocytes to mitogen as well as a reduction in IL-2 receptor expression in athletes involved in long-term training compared to healthy controls. In a recent study, Rall et al. (179) reported that 12 weeks of progressive resistance strength training did not affect immune function, e.g., lymphocyte subsets, mitogenesis, cytokine production (IL-1β, IL-2, IL-6, TNF-α) or delayed type hypersensitivity response in young or elderly healthy subjects or subject with rheumatoid arthritis. Despite reports on the immunosuppressive effect of vigorous and exhaustive exercise on mitogenesis and cytokine production, the activity and the number of NK cells have been reported to increase with exercise. For example, a study by Fiaterone et al. (168) on the effect of cycling exercise on NK cell activity in peripheral blood cells in young and elderly women showed that exercise resulted in a significant increase in the activity and the number of NK cells in both young and elderly subjects (168). In addition, they found that the NK responsiveness to recombinant IL-2 stimulation was similar between the two age groups. Nieman et al. (180) compared the effect of a 12-week exercise training program on the NK cell activity in young and elderly women. They found that the NK activity was significantly increased in the exercised young and elderly women compared to the age-matched controls. Another study involving 16 weeks of aerobic training with elderly women found that the NK activity was significantly increased in the exercised group when compared with an age-matched sedentary control (163). In response to a strenuous treadmill exercise, both

young and elderly subjects experienced a significant increase in NK activity compared with the age-matched controls. These studies indicate that in response to acute physical training, there is a transient increase in NK activity, and this is largely due to changes in the proportion of NK cells in the tested populations rather than to a change in the activity of individual cells.

CONCLUDING REMARKS

For over half a century it has been known that reduction of food intake extends the life span of rodents. Dietary restriction has received particular attention during the last two decades, perhaps because it may provide a model to study the mechanisms responsible for aging. Dietary restriction is the most efficacious intervention method known thus far that increases longevity, reduces pathology, and enhances the immune response in laboratory animals. Although the mechanism by which dietary restriction alters immunosenescence remains unclear, we have speculated that it mediates its effect by altering gene expression, e.g., expression of IL-2 gene, at the level of transcription. At the present time, it is unclear how dietary restriction alters gene expression at the level of transcription. A recent study from our laboratory supports the view that the mechanism of dietary restriction involves changes in the activities of a transcription factor, i.e., NFAT that plays a predominant role in the regulation of IL-2 transcription.

Intervention in the aging immune system by various experimental manipulations has provided immunogerontologists with the opportunity to examine the basic mechanism underlying the aging immune system. In recent years, numerous studies have been reported on the effect of hormonal or exercise therapy on the aging immune system. Some of the age-related declines in immune function have been linked to the decline in the plasma levels of the androgenic hormone DHEA or the pineal hormone MLT that occurs with advancing age. The decrease in the level of these hormones with age has been shown to be inversely correlated with the increase in the age-associated diseases such as cancer. Thus, the observation that low circulatory levels of DHEA or MLT are associated with immunosenescence has provided a rationale for hormone replacement therapy and some but not all the reported studies indicate that DHEA treatment or MLT therapy enhances the immune function and reverses the immunosenescence in mice. These studies have been conducted mainly in mice, and in some cases, lack of appropriate controls or measurements confirming the observed effects are due to DHEA/S or MLT. Thus, although these reports are encouraging, further studies are needed to confirm the immunostimulatory effect of these compounds in old experimental animals and elderly humans.

In spite of the common belief that physical activity (exercise) improves resistance to diseases, reports on the effect of exercise on lymphocyte function are conflicting and not comparable. This might be due to differences in the exercise protocol, an appropriate non-exercise control group, the intensity and duration of exercise, and the age of subjects investigated. In general, moderate exercise has been shown to enhance certain lymphocyte function. However, strenuous and vigorous exercise results in immunosuppression. The immunosuppressive effect of exercise has been associated with an increase in the stress response, i.e., the up-regulation of glucocorticoids, which are known to suppress immune function. Although considerable progress as well as controversy has been generated regarding the anti-aging and anti-immunosenescence role of DHEA, MLT, and exercise, a definitive conclusion on the therapeutic potential of these methods in retarding/reducing immunosenescence should await further confirmation by other investigators. Long-term and well-controlled studies are needed to gain better insight into the possible implications of hormonal or exercise therapy on aging and the mechanisms of action on altering immunosenescence.

ACKNOWLEDGMENTS

Thanks are expressed to Drs. G. Fernandes and H. Van Remmen for critically reviewing the manuscript. The excellent secretarial assistance of Ms. Erin K. Patterson is acknowledged. This work was supported in part by grants from the National Institute on Aging and a grant from the Nathan Shock Aging Center.

REFERENCES

1. Makinodan, T. and Kay, M.B.: Age influence on the immune system. Adv. Immunol., 29:287-296, 1980.
2. Pahlavani, M. A.: Immunological aspects of aging. Drugs of Today, 23:611-624, 1987.
3. Thoman, M. L. and Weigle, W.O.: The Cellular and Subcellular Bases of Immunosenescence. Adv. Immunol., 46:221-237, 1987.
4. Murasko, D. M. and Goonewardene, I.M.: T-Cell Function in Aging: Mechanisms of Decline. Ann. Rev. Gerontol., 10:71-88, 1990.
5. Miller, R. A.: Aging and immune function. Int. Rev. Cytol., 124:187-193, 1991.
6. Hirokawa, K.: Autoimmunity and Aging. In J.M. Cruse and R.E.J. Lewis (eds.), Autoimmunity: Basic Concepts: Systemic and Selected Organ-Specific Diseases, Karger, Basel, pp. 251-288, 1985.
7. Hirokawa, K.: Aging and the immune system. In A.M. Kligman and Y. Takase (eds.), Cutaneous Aging, University of Tokyo Press, pp. 61-78, 1988.
8. Walford, R. L.: Immunologic theory of aging: Current status. FASEB J., 33:2020., 1974.
9. Fabris, N., Pierpaoli, W., and Sorkin, E.: Lymphocytes, Hormones, and Aging. Nature, 240:557-559, 1972.

10. Hirokawa, K., Sato, K., and Makinodan, T.: Restoration of impaired immune function in aging animals. V. Long-term immunopotentiating effects of combined young bone marrow and new born thymus grafts. Clin. Immunol. Immunopathol., 22: 297-304, 1982.

11. Astle, C.M. and Harrison, D.E.: Effects of marrow donor and recipient age on immune responses. J. Immunol., 132:673-677, 1984.

12. Hirokawa, K.: Reversing and restoring immune functions. Mech. Ageing Dev., 93:119-124, 1997.

13. Pahlavani, M.A. and Richardson, A.: The effect of age on the expression of interleukin-2. Mech. Ageing Dev., 89:125-154, 1996.

14. Effros, R.B., Casillas, A., and Walford, R.L.: The effect of thymosin on immunity to influenza in aged mice. Aging Immunol. Infect. Dis., 1:31-40, 1988.

15. Goso, C., Frasca, D., and Doria, D.: Effect of synthetic thymic humoral factor (THF-γ2) on T cell activities in immunodeficient aging mice. Clin. Exp. Immunol., 87:346-351, 1992.

16. Daynes RA, Araneo BA.: Prevention and reversal of some age-associated changes

    in immunologic responses by supplemental dehydroepiandrosterone sulfate therapy. Aging: Immunol. & Infect. Dis., 3:135-153., 1992.

17. Araneo BA, Woods ML, II, Daynes RA.: Reversal of the immunosenescent phenotype by dehydroepiandrosterone: Hormone treatment provides an adjuvant effect on the immunization of aged mice with recombinant hepatitis B surface antigen. J. Infect. Dis., 167: 830-840, 1993.

18. Maestroni, G.J, Conti, A., and Pierpaoli, W.: Pineal melatonin, its fundamental immunoregulatory role in aging and cancer. Ann. Ny. Acad. Sci., 521:140-148, 1988.

19. Caroleo, M.C., Frasca, D., Nisticok, G, and Doria, G.: Melatonin as immunomodulator in immunodeficient mice. Immunopharmacol., 23:81-89, 1992.

20. Weindruch, R. H., Kriste, J.A., Naeim, F., Mullen, B.G., and Walford, R.L.: Influence of weaning-initiated dietary restriction on responses of T cell mitogens and on splenic T cell levels in a long-lived F1-hybrid mouse strain. Exp. Gerontol., 17:49-64, 1982.

21. Fernandes, G.: Nutritional Factors: Modulating effects on immune function and aging. Pharmacol. Rev., 36:123S-129S, 1984.

22. Pahlavani, M.A., Cheung, H.T., Chesky, J.A. and Richardson, A.: Influence of exercise on the immune function of rats of various ages. J. Appl. Physiol., 64:1997-2001, 1988.

23. Nasrullah, I. And Mazzeo, R.S.: Age-related immunosenescence in Fischer 344 rats: Influence of exercise training. J. Appl. Physiol., 73:1932-1938, 1992.

24. Venkatraman, J.T., and Fernandes, G.: Exercise, immunity and aging. Aging Clin. Exp., 9:4256, 1997.

25. Chandra, R.K.: Effect of vitamin and trace-element supplementation on immune responses and infection in elderly subjects. Lancet, 340:1124-1127, 1992.

26. Bogden, J.D., Bendich, A., Kemp, F.W., Bruening, K.S., Shurnick, J.H., Denny, T., Baker, H., and Louria, D.B.: Daily micronutrient supplements enhance delayed hypersensitivity skin test responses in older people. Am. J. Clin. Nutr., 60:437-447, 1994.

27. Meydani, S.N., Meydani, M., Blumberg, J.B., Leka, L.S., Siber, G., Loszewski, R., Thompson, C., Pedrosa, M.C., Diamond, R.D., and Stollar, B.D.: Vitamin E supplementation and in vivo immune response in healthy elderly subjects. A randomized controlled trial. JAMA 277:1380-1386, 1997.

28. Masoro, E. J., Shimokawa, I., Higami, Y., McMahan, C. A. and Yu, B.P.: Temporal pattern of food restriction not a factor in the restoration of aging process by dietary restriction. J. Gerontol., 50:B48-B53, 1995.

29. Richardson A.: The effect of age and nutrition on protein synthesis by cells and tissues from mammals, in *CRC Handbook of Nutrition and Aging*. Watson, R.R., Ed., CRC Press, Boca Ration, FL, 31, 1985.

30. Weindruch, R.H., Kriste, J.A., Cheney, K.E., and Walford, R.L.: Influence of controlled dietary restriction on immunologic function and aging. Fed. Proc., 38:2007-2016, 1979.

31. Regelson, W., Loria, R., and Kalami, M.: Dehydroepiandrosterone (DHEA) – the "Mother Steroid". I. Immunologic action. Ann. N. Y. Acad. Sci., 719, 553-563, 1994.

32. Regelson, W., Kalimi, M., and Loria, R.: DHEA: some thoughts as to its biologic and clinical action. In: Kalimi M, Regelson W. eds. The Biologic role of Dehydroepi-androsterone (DHEA). Walter de Guyter: New York, pp. 405-445, 1990.

33. Maestroni, G.J, Conti, A., and Pierpaoli, W.: Pineal melatonin, its fundamental immunoregulatory role in aging and cancer. Ann. Ny. Acad. Sci., 1988, 521:140-148, 1988.

34. Maestroni, G., and Conti, A.: Melatonin and the immune-hematopoietic system. Theapeuric and adverse pharmacological correlates. Neuroimmunomodulation, 3:325-332, 1996.

35. McCay, C. M., Crowell, M.F., and Maynard, L.A.: The effect of retarded growth upon the length of life span and upon the ultimate body size. J. Nutr., 10:63-72, 1935.

36. Masoro, E. J. and McCarter, R.M.: Dietary Restriction as a Probe of Mechanisms of Senescence. Ann. Rev. Gerontol., Chapter 11:183-194, 1990.

37. Masoro, E. J.: Potential role of modulation of fuel use in the antiaging action of dietary restriction. Ann. NY Acad. Sci., 663:403-411, 1992.

38. Gerbase-Delima, M., R. K. Liu, K. E. Cheney, R. Mickey, and Walford, R.L.: Immune function and survival in the long-lived mouse strain subjected to undernutrition. Gerontologia, 21:184-193, 1975.

39. Fernandes, G., E. Yunis, J., and Good, R.A.: Influence of protein restriction on immune function in NZB mice. J. Immunol., 116:782-788, 1976.

40. Venkatraman, J. and Fernandes, G.: Modulation of age-related alterations in membrane composition and receptor-associated immune functions by food restriction in Fischer 344 rats. Mech. Ageing Dev., 63:27-44, 1992.

41. Miller, R. A.: Caloric restriction and immune function: developmental mechanisms. Aging Clin. Exp. Res., 3:395-403, 1991.

42. Gilman-Sachs, A., Kim, Y.B., Pollard, M., and Snyder, L.D.: Influence of Aging, Environmental Antigens, and Dietary Restriction on Expression of Lymphocyte Subsets in Germ-free and Conventional Lobund-Wistar Rats. J. Gerontol., 46: B101-B109, 1991.

43. Pahlavani, M. A., Cheung, H.T., Cai, N.S., and Richardson, A.: Influence of dietary restriction and aging and gene expression in the immune system of rats. In Biomedical advances in aging. A.L. Goldstein, ed. Plenum Publishing Corp., New York, p. 259-270, 1990.

44. Lindquist, S.: The heat-shock response. Ann. Rev. Biochem., 55:1151-1191, 1986.

45. Lindquist, S., Craig, E.A.: The heat-shock proteins. Ann. Rev.Genet., 22:631-677, 1988.

46. Young, D.B.: Heat shock proteins: Immunity and Autoimmunity. (review). Current Opinion in Immunology, 4:396-400, 1992.

47. Sribvastava, P.K., Udono, H., Blachere, N.E., and Li, Z.: Heat shock proteins transfer peptides during antigen processing and CLT priming (review). Immunogenetics, 39:93-98, 1994.

48. Pahlavani, M.A., Harris, M.D., Moore, S.A., and Richardson, A.: Expression of heat shock protein 70 in rat spleen lymphocytes is affected by age but not by food restriction. N. Nutr., 126:2060-2075, 1996.

49. Byun, D.S., Venkartramann, J.T., Yu, B.P., and Fernandes, G.: Modulation of antioxidant activities and immune response by food restriction in aging Fischer 344 rats. Aging Clin. Exp., Res., 7:40-48, 1995.

50. Chayoth, R., Christou, N.V., Nohr, C.W., Yale, J.F., Poussier, P., Grose, M., Montambault, M., Chan, W., and Marliss, E.B.: Immunological responses to chronic heat exposure and food restriction in rats. Am. J. Clin. Nutr., 48:361-367, 1988.

51. Goonewardene, I.M. and Murasko, D.M.: Age-associated changes in mitogen-induced lymphoproliferation and lymphokine production in the long-lived Brown-Norway rat: Effect of caloric restriction. Mech. Ageing Dev., 83:103-116, 1995.

52. Konno, A., Utsuyama, M., Kurashima, C., Kasai, M., Kimmura, S., and Hirokawa, K.: Effects of a protein-free diet or food restriction on the immune system of Wistar and Buffalo rats at different ages. Mech. Ageing and Dev., 72:183-197, 1993.

53. Pieri, C., Rechioni, R., and Moroni, F.: Food restriction in female Wistar rats. VI. Effect of reduced glutathione on the proliferative response of splenic lymphocytes from ad libitum fed and food restricted animals. Arch. Gerontol. Geriatr., 16:81-92, 1993.

54. Iwai, H. and Fernandes,G.: Immunological functions in food-restricted rats: enhanced expression of high-affinity interleukin-2 receptors on splenic T cells. Immunol. Lett., 23:125-132, 1990.

55. Fernandes, G., Venkatraman, J., Khare, A., Horbach, G.J., and Friedrichs, W.: Modulation of gene expression in autoimmune disease and aging by food restriction and dietary lipids. Proc. Soc. Exp. Biol. Med., 193:16-25, 1990.

56. Pahlavani, M.A. Harris, M.D., and Richardson, A.: The increase in the induction of IL-2 expression with caloric restriction is correlated to changes in the transcription factor NFAT. Cell. Immunol., 180:10-19, 1997.

57. Hishinuma, K., Nishimura, T., Konno, A., Hashimoto, Y., and Kimura, S.: The effect of dietary restriction on mouse T cell functions. Immunol. Lett., 17:351-359, 1988.

58. Weindruch, R. H., Kriste, J.A., Naeim, F., Mullen, B.G., and Walford, R.L.: Influence of weaning-initiated dietary restriction on responses of T cell mitogens and on splenic T cell levels in a long-lived F1-hybrid mouse strain. Exp. Gerontol., 17:49-64, 1982.

59. Weindruch, R.H., Kriste, J.A., Cheney, K.E., and Walford, R.L.: Influence of controlled dietary restriction on immunologic function and aging. Fed. Proc., 38:2007-2016, 1979.

60. Weindruch, R., Devens, B.H., Raff, H.V., and Walford, R.L.: Influence of dietary restriction and aging on natural killer cell activity in mice. J. Immunol., 130:993-996, 1983.

61. Grossmann, A., Price, L., Jinneman, J.C., Wolf, N.S., and Rabinovitch, P.S.: The effect of long-term caloric restriction on function of T cell subsets in old mice. Cell. Immunol., 131:191-204, 1990.

62. Cheney, K.E., Liu, R.K., Smith, G.S., Meredith, P.J., Michey, M.R., Walford, R.L.: The effect of dietary restriction of varying duration on survival, tumor patterns, immune function, and body temperature in B10C3F1 female mice. J. Gerontol., 38:420-430, 1983.

63. Venkatraman, J., Attwood, V.G., Turturro, A., Hart, R.W., and Fernandes, G.: Maintenance of virgin T cells and immune function by food restriction during aging in ling-lived B6D2F1 female mice. Aging: Immunol. Infec. Dis., 5:13-25, 1994.

64. Fernandes, G.: Nurtritional Factors: Modulating Effects on Immune Function and Aging. Pharmacol. Rev., 36:123S-129S., 1984.

65. Kubo, C., Johnson, B.C., Day, N.K., and Good, R.A.: Calorie Source, Calorie Restriction, Immunity and Aging of (NZB/NZW)F1 mice. J. Nutr., 114:1884-1899, 1984.

66. Fernandes, G., Friend, P., Yunis, E.J., and Good, R.A.: Influence of dietary restriction on immunological function and renal disease in (NZBXNZW)F1 mice. Proc. Natl. Acad. Sci. USA, 75:1500-1504, 1978.

67. Jung, L.K., Palladino, M.A., Calvano, S., Mark, D.A., Good, R.A., and Fernandes, G.: Effect of calorie restriction on the production and responsiveness to interleukin-2 in (NZB/NZX)F1 mice. Clin. Immunol., Immunopharmacol., 25:295-301, 1982.

68. Ellis, M.: An introduction to transcription. In Genes and Cancer. D. Carney and K. Sikora, eds. John Wiley and Sons, Chichester, UK, p. 107-118, 1990.

69. Angel, P., Allegretto, E.A., Okino, S.T., Hattari, K., Boyle, W.J., Hunter, T., and Karin,M.: Oncogene jun encodes a sequence-specific trans-activator similar to AP-1. Nature, 332:166-168, 1988.

70. Mitchell, P. J. and Tjian, R.: Transcriptional regulation in mammalian cells by sequence-specific DNA binding proteins. Science, 245:371-373, 1989.

71. Ullman, K. S., Northrop, J.P., Verweij, C.L., and Crabtree, G.R.: Transmission of Signals from the T Lymphocyte Antigen Receptor to the Genes Responsible for Cell Proliferation and Immune Function: The Missing Link. Annu. Rev. Immunol., 8:421-443, 1990.

72. Riegel, J. S., Corthesy, B., Flanagan, W.M., and Crabtree, G.R.: Regulation of the Interleukin-2 Gene. Chem. Immunol., 51:266-287, 1992.

73. Regelson, W., and Kalami, M.: Dehydroepiandrosterone (DHEA) – the Multifunctional Steroid. II. Effect on the CNS, cell proliferation, metabolic and vascular, clinical and other effects. Mechanism of Action. Ann. N. Y. Acad. Sci., 719, 564-575, 1994.

74. Schwartz, A.G.: Inhibition of spontaneous breast cancer formation in female (C3H-A^{v}y/A) mice by long term treatment with dehydroepiandrosterone. Cancer Res., 39: 1129-1132, 1979.

75. Parker C.R, and Schuster, M.W: Effects of syphilis infection on adrenocortical function in man. Proc. Soc. Exp. Biol. Med., 197:165-167, 1991.

76. Moolenar, A.J., Van Seters, A.P.: Gas-chromatographic determination of steroids in the urine of patients with Cushing's syndrome. Acta Endocrinology (Copenhagen), 67:303-315, 1971.

77. Keutman, E.H., Mason W.B.: Individual urinary 17-ketosteroids of healthy persons determined by gas-chromatograph-biochemical and clinical consideration. J. Clin. Endocrinol., 27:406-420, 1967.

78. Loria, R.M., Inge, T.H., Cook ,S.: Protection against acute lethal viral infections with the native steroid dehydroepiandrosterone (DHEA). J. Med, Virol., 26: 301-314, 1988.

79. Loria, R.M., Regelson, W., Padget, D.A.: Immune response facilitation and resistance to viral and bacterial infections with DHEA. In: The Biologic Role of Dehydroepiandrosterone (DHEA). Kalimi, M., Regelson, W. (Eds.). Walter de Gruyter, New York, pp. 107-130, 1990.

80. Ben-Nathan D, Feuerstein G.: The influence of cold and isolation stress on resistance of mice to West Nile virus encephalitis. Experientia, 46:285-290, 1990.

81. Rasmussen, D.R., Martin, E.G., Arrowood, M.J.: Effect of dexamethasone and dehydroepiandrosterone in immunosuppressed rats infected with *Crytosporidium parvum.* J. Protozool., 38:157-159, 1991.

82. Araneo, B.A., Shelby, J., Li, G.Z.: Administration of dehydroepiandrosterone to burned mice preserves normal immunologic competence. Arch. Surg., 128: 318-325, 1993.

83. Daynes, R.A., Dudley, D.J., Araneo, B.A.: Regulation of murine lymphokine production *in vivo.* II. Dehydroepiandrosterone is a natural enhancer of IL-2 synthesis by helper T-cells. Eur. J. Immunol., 20:793-801, 1990.

84. Padgett, D.A. and Loria ,R.M.: In vitro potentiation of lymphocyte activation by dehydroepiandrosterone, androstenediol, and androstenetriol. J. Immunol., 153: 1544-1552, 1994.

85. Risdon, G., Cope, J., and Bennett, M.: Mechanisms of chemoprevention by dietary dehydroepiandrosterone: Inhibition of lymphopoiesis. Am. J. Pathol., 136:759-769, 1990.

86. Pahlavani, M.A. and Harris, M.D.: Effect of dehydroepiandrosterone on mitogen-induced lymphocyte proliferation and cytokine production in young and old F344 rats. Immunol. Lett., 47:9-14, 1995.

87. Blauer, K.L, Poth, M., Rogers, W.M., and Bernton, E.W.: Dehydroepiandrosterone antagonizes the suppressive effects of dexamethasone on lymphocyte proliferation. Endocrinol., 129:3174-3179, 1991.

88. Risdon, G.: Differential effects of DHEA on murine lymphopoiesis and myelopoiesis. Exp. Hematol., 19:128-131, 1991.

89. Matsunaga, A., Miller, B.C., Cottam, G.L.: Dehydroepiandrosterone prevention of autoimmune disease in NZB/WF1 mice: lack of an effect on associated immunological abnormalities. Biochem. Biophys. Acta., 992: 265-271, 1989.

90. Risdon, G., Cope, J., and Bennett, M.: Mechanisms of chemoprevention by dietary dehydroepiandrosterone: Inhibition of lymphopoiesis. Am. J. Pathol., 136:759-769, 1990.

91. Araneo, B.A., Dowell, T., Diegel, M., and Daynes, R.A.: Dehydroepiandrosterone exerts a depressive influence on the production of interleukin-4 (IL-4), IL-5, and interferon-gamma, but no IL-2 by activated murine T cells. Blood, 78:688-699, 1991.

92. Garg, M. and Bonada, S.: Reversal of age-associated decline in immune response to Pnu-Immune Vaccine by supplementation with the steroid hormone dehydroepiandrosterone. Infec. & Immunol., 61:2238-2241, 1993.

93. Weinduruch, R., McFeeters,G., and Walford, R.L.: Food intake reduction and immunologic alterations in mice fed dehydroepiandrosterone. Exp. Gerontol., 19:295-304, 1984.

94. Wiedmeier, S.E., Mu, H., Araneo, B.A., and Daynes, R.A.: Age and microenvironment-associated influences by platelet-derived growth factor on T cell function. J. Immunol., 152:3417-3425, 1994.

95. Daynes, R.A., Araneo, B.A., Ershler, W.B., Maloney, C., Li, G., and Ryu, S.: Altered regulation of IL-6 production with normal aging: Possible linkage to the age-associated decline in dehydroepiandrosterone and its sulfated derivative. J. Immunol., 150:5219-5230, 1993.

96. May, M., Holmes, E., Rogers, W., and Poth, M.: Protection from glucocorticoid induced thymic involution by dehydroepiandrosterone. Life Science, 46:1627-1631, 1990.

97. Dyner, T.S., Lang, W., Geaga, L., Golub, A., Sites, D., Winger, E., Galmarini, M., Masterson, J., and Jacobson, M.A.: An open-label dose-escalation trial of oral dehydroepiandrosterone tolerance and pharmacokinetics in patients with HIV disease. J. Acquired Immune Deficiency Syndromes, 6:459-465, 1993.

98. Yang, J., Schwartz, A., and Henderson, E.: Inhibition of HIV-1 latency reactivation by dehydroepiandrosterone (DHEA) and an analog of DHEA. AIDS Res. And Human Retroviruses, 9:747-754, 1993.

99. Suzuki, T., Suzuki, N., Daynes, R.A.: Dehydroepiandrosterone enhances IL-2 production and cytotoxic effector function of human T-cells. Clin. Immunol. Immunopathol., 61:202-211, 1991.

100. Khorram, O., Vu, L., and Yen, S.: Activation of immune function by dehydroepiandrosterone (DHEA) in age-advanced men. J. Gerontol., 52A:M1-M7, 1997.

101. Maestroni, G.J, Conti, A.: Melatonin in relation to the immune system. In: Yu,H.S., Reiter, R.J. (eds), Melatonin: Biosynthesis, Physiological Effects and Clinical Applications. Boca Raton, CRC Press, pp. 290-306, 1993.

102. Maestroni, G.J.: The immunoendocrine role of melatonin. J. Pineal. Res., 14:1-10, 1993.

103. Pahlavani, M.A.: Role of melatonin in immunity, aging, and cancer: therapeutic intervention. Drugs of Today, 22:25-39, 1997.

104. Maestroni, G.M. and Pierpaoli, W.: Pharmacologic control of the hormonally mediated immune response. In: R. Ader (Ed.) Psychoneuroimmunology. Academic Press, New York, pp. 405-425, 1981.

105. Maestroni, G.M., Conti, A., and Pierpaoli, W.: Role of the pineal gland in immunity: Circadian synthesis and release of melatonin modulates the antibody response and antagonizes the immunosuppressive effects of corticosterone. J. Neuroimmunol.,13:19-30, 1986.

106. Caroleo, M.C., Doria, G., and Nistico, G.: Melatonin restores immunodepression in aged and cyclophosphamide-treated mice. Ann. Ny. Acad. Sci., 719:343-352, 1994

107. Kuci, S., Becker, J., and Veit, G.: Circadian variations of the immunomodulatory role of the pineal gland. Neuroendocrinol., Lett., 10:65-73, 1983.

108. Becker, J., Veit, G., Handgretinger, R., et al. Circadian variations in the immunomodulatory role of the pineal gland. Neuroendocrinol. Lett.,10:65-72, 1998.

109. Vermeulen, M., Palermo, M., and Giordano, M.: Neonatal pinealectomy impairs murine antibody-dependent cellular toxicity. J. Neuroimmunol., 43:97-101, 1993.

110. Del Gobbo, V., Libri, V., Villani, N., Callio, R., and Nistico, G.: Pinealectomy inhibits interleukin-2 production and natural killer activity in mice. Int. J. Immunopharmacol., 11:567-577, 1989.

111. Fraschini, F., Scaglione, F., Franco, P., Demartini, G., Lucini, V., and Stankov, B.: Melatonin and Immunity. Acta. Oncol., 5:775-776, 1990.

112. Maestroni, G. and Conti, A.: The pineal neurohormone melatonin stimulates activated CD4+, Thy-1+ cells to release opioid agonists with immunoenhancing and anti-stress properties. J. Neuroimmunol., 28:167-176, 1990.

113. Mocchegiani, E., Bulian, D., Santarelli, L., Tibaldi, A., Muzzioli M., Pierpaoli, W., and Fabris, N.J.: The immuno-reconstituting effect of melatonin or pineal grafting and its relation to zinc pool in aging mice, Neuroimmunol., 53:189-196, 1994.

114. Ben-Nathan D., Maestroni, G.M., and Conti, A.: Protective effect of melatonin in mice infected with encephalitis viruses. Arch. Virol., 140:223-230, 1995.

115. Pierpaoli, W. and Regelson, W.: Pineal control of aging: Effect of melatonin and pineal grafting on aging mice. Proc. Natl. Acad .Sci. USA, 91:787-791, 1994.

116. Provinciali, M., Stefano, G., Bulian, D., Tibaldi, A., and Fabris, N.: Effect of melatonin and pineal grafting on thymocyte apoptosis in aging mice, Mech. Ageing & Dev., 90:1-9, 1996.

117. Maestroni, G.J.: T-helper-2 lymphocytes as a peripheral target of melatonin. J. Pineal Res., 18:84-89, 1995.

118. Csaba, G., and Barath, P.: Morphological changes of thymus and the thyroid gland after postnatal extirpation of the pineal body. Endocrinol. Exp., 9:59-67,1975.

119. Maestroni, G.J, Conti, A., and Pierpaoli, W.: Role of the pineal gland in immunity. III. Melatonin antagonizes the immunosuppressive effect of acute stress via an opiatergic mechanism, Immunology, 63:465-476, 1988.

120. Pioli, C., Caroleo, M.C., Nistico, G., and Doria, D.: Melatonin increases antigen presentation and amplifies specific and nonspecific signals for T cell proliferation. Int. J. Immunopharmacol., 15:463-469, 1993.

121. Sainz, R.M., Mayo, J.C., Urfa, H., Kotler, M., Antolin, A., Fodriguez, C., and Pelaex, A.: The pineal neurohormone melatonin prevents in vivo and in vitro apoptosis in thymocytes. J. Pineal Res., 19:178-188, 1995.

122. Pahlavani, M.A. and Harris, M.D.: In vitro effects of melatonin on mitogen-induced lymphocyte proliferation and cytokine expression in young and old rats. Immunopharmacol. & Immunotoxicol., 19:327-337, 1997.

123. Vaughan, M.K., Hubbard, G.B., Champeny, T.H., Little, J.C., and Reiter, R.J.: Splenic hypertrophy and extramedullary hematopoiesis induced in male Syrian hamsters by short photoperiod or melatonin injections reversed by melatonin pellets or pinealectomy. Am. J. Anat., 179:131-136, 1978.

124. Morrey, M.K. McLachlan, J.A., Serkin, D.C., and Bakouche, O.: Activation of human monocytes by the pineal neurohormone melatonin. J. Immunol.,153:2671-2680, 1994.

125. Lissoni, P., Pittalis, S., Brivio, F., Tisi, E., Rovelli, F., Ardizzovia A., Barni, S., Tanicini, G., Giudici, G., Biondi, A., Conti, A., and Maestroni, G.: In vitro modulatory effects of interleukin-3 on macrophage activation induced by interleukin-2. Cancer, 71:2976-2981, 1993.

126. Vijayalaxmia, R., Reiter, R. J., and Meltz, M.L.: Melatonin protects human blood lymphocytes from radiation-induced chromosome damage. Mutation Res., 346:23-31, 1995.

127. Karasek, M., Liberski, P., Radek, J., and Bartkowiak, J.: Influence of melatonin on the proliferation of hepatoma cells in the Syrian hamster: In vivo and in vitro study, Rovelli, F., Cattaneo, G., Archili, C., and Barni, S.: Serum interleukin-2 levels in relation to the neuroendocrine status in cancer patients. Br. J. Cancer, 62:838-839, 1990.

129. Das Gupta, T.K.: Influence of pineal gland on the growth and spread of melanoma in hamsters. Cancer Res., 19:83-84, 1968.

130. Stanberry, L.R., Das Gupta, T.K., and Beattle, C.W.: Photoperiodic control of melanoma growth in hamsters: Influence of pinealectomy and melatonin. Endocrinology, 113:469-475, 1983.

131. Tamarkin, L., Cohen, M., and Roselle, D.: Melatonin inhibition and pinealectomy enhancement of 7,12-dimethylbenzanthracene induced mammary tumor in therats. Cancer Res 1981, 41:432-436, 1981.

132. Blask, D.E. and Hill, S. M.: Melatonin and Cancer: Basic and clinical aspects. In: Miles A., Philbrick, D.R., Thompson, C. (Eds.) Melatonin: Clinical Aspects. Oxford University, Oxford U.K, pp. 128-173, 1988.

133. Lissoni, P., Barni, S., Crispino, S., Tancini, G., and Fraschini, F.: Endocrine and immune effects of melatonin therapy in metastatic cancer patients. Eur. J. Cancer Clin. Oncol., 25:789-792, 1989.

134. Conti, A. and Maestroni, G.M.: The clinical neuroimmunotherapeutic role of melatonin in oncology. J. Pineal Res., 10:103-110, 1995.

135. Lissoni, P., Barni, S., Gazzaniga, M., Ardizzoia, A., Rovelli, F., Brivio, F., and Tancini, G.: Efficacy of the concomitant administration of the pineal hormone melatonin in cancer immunotherapy with low-dose IL-2 in patients with advanced solid tumors who had progressed on IL-2 alone. Oncology, 51:344-347, 1994.

136. Lissoni, P., Tisi, E., Barni, S.: Biological and clinical results of a neuroimmuno-therapy with IL-2 and the pineal hormone melatonin as a first line treatment in advanced non-small cell lung cancer. Br. J. Cancer, 66:155-158, 1992.

137. Neri, B., Fiorelli, C., Moroni, F.: Modulation of human lymphoblastoid interferon activity by melatonin in metastatic renal cell carcinoma. Cancer, 73:3015-5019, 1994.

138. Lissoni, P., Barni, S., Ardizzoia, A., Paolorossi, F., Crispino, S.: Randomized study with the pineal hormone melatonin versus supportive care alone in advanced nonsmall cell lung cancer resistant to first-line chemotherapy containing cisplatin. Oncology, 49: 336-339, 1992.

139. Lissoni, P., Barni, S., Rovelli, F., Brivio, F., Ardizzoia, A., Tancini, G., Conti, A, and Maestroni, G.J: Neuroimmunotherapy of advanced solid neoplasms with single evening subcutaneous injection of low-dose IL-2 and melatonin: Preliminary results. Eur. J. Cancer., 29A:185-189, 1993.

140. Lissoni, P., Barni, S., Tancini, G.: Immunotherapy with subcutaneous low-dose IL-2 and the pineal indole melatonin as a new effective therapy in advanced cancers of the digestive tract. Br. J. Cancer, 67:1404-1407, 1993.

141. Lissoni, P., Brivio, F., Brivio, O., Fumagalli, L., Gramazio, F., Rossi, M., Emanuelli, G., Alderi, G., and Lavorato, F.: Immune effects of preoperative immunotherapy with high-dose subcutaneous interleukin-2 versus neuroimmunotherapy with low-dose interleukin-2 plus the neurohormone melatonin in gastrointestinal tract tumor patients. J. Biol. Regul. Homeost. Agents, 9:31-33, 1995.

142. Lissoni, P., Barni, S., Ardizzoia, A., Tancini, G., Conti, A, and G.J. Maestroni, G.M.: A randomized study with the pineal hormone melatonin versus supportive care alone in patients with brain metastases due to solid neoplasms. Cancer, 73:699-701, 1994.

143. Lissoni, P., Barni, S., Tancini, G., Mainini, E., Piglia, F., and Maestroni, G.M.: Immunoendocrine therapy with low-dose subcutaneous interleukin-2 plus melatonin locally advanced or metastatic endocrine tumors. Oncology, 52:163-166, 1995.

144. Lissoni, P., Brivio, F., Ardizzoia, A., Tancini, G., and Barni, S.: Subcutaneous therapy with low-dose IL-2 plus neurohormone melatonin in metastatic gastric cancer patients with low performance status. Tumori, 79:410-404, 1993.

145. Lissoni, P., Barni, S., Rovelli, F.: Neuroendocrine effects of subcutaneous IL-2 injection in cancer patients. Tumori, 77:212-215, 1991.

146. Lissoni, P., Barni, S., Tancini, G.: A randomized study with subcutaneous low-dose IL-2 alone vs. IL-2 plus the pineal hormone melatonin in advanced solid neoplasm other than renal cancer and melanoma. Br. J .Cancer, 69:196-199, 1994.

147. Lissoni, P., Meregalli, S., Fossati, V.: A randomized study of immunotherapy with low-dose subcutaneous IL-2 plus melatonin vs. chemotherapy with cisplatin and etoposide as first-line therapy for advanced non-small lung cancer. Tumori, 80:464-467, 1994.

148. Barni, S., Lissoni, P., Cazzaniga, M.: Neuroimmunotherapy with subcutaneous low-dose IL-2 and the pineal hormone melatonin as a second-line treatment in metastatic colorectal carcinoma. Tumori, 78:383-387, 1992.

149. Turek, F.W.: Melatonin hype hard to swallow. Nature, 379:295-296, 1996.

150. Cunningham, D.A., and Hill, J.S.: Effects of training on cardiovascular response to exercise. J. Appl. Physiol., 39:891-895, 1975.

151. Mahan, M.P. and Young, R.M.: Immune parameters of untrained or exercise-trained rats after exhaustive exercise. J. Appl. Physiol.,66:282-287, 1989.

152. Shu, J., Stevenson, J.R., and Zhou, Z.: Modulation of cellular immune responses by cold water stress in the rat. Develop. Comp. Immunol., 17:357-371, 1993.

153. Ferry, A., Weill, B.L., and Rieu, A.M.: Immunomodulations induced in rats by exercise on a treadmill. J. Appl. Physiol., 69:1912-1915, 1990.

154. Fernandes, G, Rozek, M., and Troyer, D.: Reduction of blood pressure and restoration of T-cell immune function in spontaneously hypertensive rats by food restriction and/or by treadmill exercise. J. Hypertension, 4:S469-S474, 1986.

155. Tharp, G.D. and Preuss, T.L.: Mitogenic response of T lymphocytes to exercise training and stress. J. Appl. Physiol., 70:2535-25-38, 1991.

156. Simpson, J.R. and Hoffman-Goetz, L.: Exercise stress and murine natural killer cell function. Proc. Soc. Exp. Biol. Med., 195:129-135, 1990.

157. Ortega, E., Forner, M.A., Barriga, C., and De La Fuente, M.: Effect of age and of swimming-induced stress on the phagocytic capacity of peritoneal macrophages from mice. Mech. Ageing Dev., 70:53-63, 1993.

158. Filteau, S.M., Menzies, R.A., Kaido, T.J., O'Grady, M.P., Gelderd, J.B., and Hall, N.R.: Effects of exercise on immune functions of undernourished mice. Life Sci., 51:565-574, 1992.

159. De La Fuente, M., Fernandez, M.D., Miguel, J., and Hernanz, A.: Changes with aging and physical activity in ascorbic acid content and proliferative response of murine lymphocytes. Mech. Ageing Dev., 65:177-186, 1992.

160. Hoffman-Goetz, L., Keir, R., Thorne, R., Houston, M.E., and Young, C.: Chronicexercise stress in mice depresses splenic T lymphocyte mitogenesis in vitro. Clin. Exp. Immunol., 66:551-557, 1986.

161. Conn, C.A., Rozak, W.E., Tooten, P.J., Gruys, E., Borer, K.T., and Kluger, M.J.: Effect of voluntary exercise and food restriction in response to lipopolysaccharide in hamsters. J. Appl. Physiol., 78:466-477, 1995.

162. Tomasi, T.B., Thudeau, F.B., Czerwinski, D., and Erredge, S.: Immune parameters in athletes before and after strenuous exercise. J. Clin. Immunol., 2:173-177, 1982.

163. Crist, D.M., Mackinnon, L.T., Thompson, R.F., Atterbon, H.A., and Egan, P.A.: Physical exercise increases natural cellular-mediated tumor cytotoxicity in elderly women. Gerontol., 35:66-71, 1989.

164. Papa, S., Vitale, M., Mazzotti, G., Neri, L., Monti, G., and Manzoli, F.: Impaired lymphocyte stimulation induced by long-term training. Immunol. Lett., 22:29-33, 1989.

165. MacNeil, B., Hoffman-Goetz, L., Kendall, A., Houston, M., and Arumagam, Y.: Lymphocyte proliferation responses after exercise in men: fitness, intensity, and duration. J. Appl. Physiol., 70:179-185, 1991.

166. Eskola, J., Ruuskanen, E., Soppi, E.: Effect of sport stress on lymphocyte transformation and antibody production. Clin. Exp. Immunol., 32:339-345, 1978.

167. Lewicki, R., Tchorzewski, H., Majewska, E., Nowak, Z., and Baj, Z.: Effect of maximal physical exercise on T lymphocyte subpopulations and on interleukin-1 (IL-1) and interleukin-2 (IL-2) production in vitro. Int. J. Sports Med., 9:114-117, 1988.

168. Fiatarone, M.A., Morley, J.E., Bloom, E.T., Benton, D., Solomon, G.F., and Makinodan, T.: The effect of exercise on natural killer cell activity in young and old subjects. J. Gerontol., 44:M37-45, 1989.

169. Ferry, A., Picard, F., Duvallet, A., Weill, B., and Rieu, M.: Changes in blood leukocyte populations induced by acute maximal and chronic submaximal exercise. Eur. J. Appl. Physiol., 59:435-442, 1990.

170. Hanson, P.G., and Flaherty, D.K.: Immunological responses to training in conditioned runners. Clin. Sci., 60:225-228, 1981.

171. Liu, Y.G., and Wang, S.Y.: The enhancing effect of exercise on production of antibody to Salmonella typhi in mice. Immunol. Lett., 14:117-120, 1987.

172. Peters, B.A., Sothmann, M., and Wehrenberg, W.B.: Blood leukocyte and spleen lymphocyte immune responses in chronically physically active and sedentary hamsters. Life Sci., 45:2238-2245, 1989.

173. Hedfors, E., Holm, G., and Ohnel, B.: Variations of blood lymphocytes during work studied by cell surface markers, DNA synthesis, and cytotoxicity. Clin. Exp. Immunol., 24:328-335, 1976.

174. Hoffman-Goetz, L., Keir, R., Thorne, R., Houston, M.E., and Young, C.: Chronicexercise stress in mice depresses splenic T lymphocyte mitogenesis in vitro. Clin. Exp. Immunol., 66:551-557, 1986.

175. Oshida, Y., Yamanouchi, K., Hayamizu, U., and Sato, Y.: Effect of acute physical exercise on lymphocyte subpopulations in trained and untrained subjects. Int. J. Sports Med., 9:137-140, 1988.

176. Fitzgerald, L.: Exercise and immune system. Immunol. Today, 11:337-339, 1988.

177. Khansari, D.N., Murgo, A.J., and Faith, R.E.: Effects of stress on immune system. Immunol. Today, 11:170-175, 1990.

178. Hoffman-Goetz, L., Thorne, R.J., and Houston, M.E.: Splenic immune responses following treadmill exercise in mice. Can. J. Physiol. Pharmacol., 66:1405-1419, 1988.

179. Rall, L.C., Roubenoff, R., Cannon, J.G., Abad, L.W., Dinarello, C.A., and Meydani, S.N.: Effects of progressive resistance training on immune response in aging and chronic inflamation. Med. Sci. Sports Exerc. 28:1356-1365, 1996.

180. Nieman, D.C., Henson, D.A., and Gusewitch, G.: Physical activity and immune function in eldely women. Med. Sci. Sports Exer., 25:823-831, 1993.